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Neonatal Hyperbilirubinemia Associated With Southeast Asian Ovalocytosis

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We report, herein, an infant who is twin A of a dizygotic twin, with premature birth and both twins having hemoglobin (Hb) E heterozygosity. Twin A who had Southeast Asian ovalocytosis (SAO) developed neonatal jaundice at the age of 2 days and needed phototherapy at the age of 3 days. The microbilirubin level was rapidly rising up to 535.2 μmol/L (31.3 mg/dl) with the hematocrit value of 38% at the age of 4 days prior to exchange blood transfusion. Exchange blood transfusion was done by 220 ml of O, Rh positive packed red blood cell reconstituted with 180 ml of O, Rh positive fresh plasma to lower the bilirubin level. Twin A received phototherapy from about 8 hr prior to exchange blood transfusion until 3 days later. Twin B, who did not have SAO, developed neonatal hyperbilirubinemia and needed only phototherapy. Twin A received a deletion of 27 base-pairs in the erythroid band 3 gene and Hb E heterozygosity from his father. Am. J. Hematol. 60:136–139, 1999. © 1999 Wiley-Liss, Inc.

Key words: neonatal hyperbilirubinemia; Southeast Asian Ovalocytosis; prematurity

INTRODUCTION

Southeast Asian ovalocytosis (SAO) is widespread in certain ethnic groups of Malaysia, Papua New Guinea, Philippines, Indonesia [1] and Thailand. SAO is an asymptomatic trait characterized by the presence of oval red blood cells (RBCs) many of which contain one or two transverse ridges or a longitudinal slit [1]. The mode of transmission is an autosomal dominant [1]. The homozygous state is thought to be lethal in utero [2]. The red cell membrane is markedly increased in rigidity and resistance to invasion by several malarial parasite strains. The underlying molecular defect in SAO involves a deletion of nine codons (codons 400–408) in the erythroid band 3 gene [3]. In the great majority of individuals it is not associated with anemia or a significant degree of increased hemolysis [4]. Neonatal hyperbilirubinemia associated with SAO has been reported in 1971 [5], but only a few cases. Herein we report a boy who had neonatal hyperbilirubinemia due to SAO and prematurity and needed exchange blood transfusion and phototherapy.

CASE REPORT

A Thai male infant who was twin A was born at 36 weeks of gestation by cesarean section to a 32-year-old woman with gravida 1, para 0, abortion 0. Antenatal care was done since 2 months of pregnancy. The mother did not undergo amniocentesis or other invasive obstetrical monitoring during the pregnancy. The mother's pregnancy had been uncomplicated until there was abnormal fetal heart rate in one twin after a nonstress test and cesarean section was performed. The apgar scores in twin A were 9, 10 and twin B were 8, 10 at 1 and 5 min, respectively. The birth weight of twin A was 2,200 g and twin B, who is female, was 2,210 g. The birth hemoglobin (Hb) and hematocrit (Hct) values for both infants were not done.

Abbreviations used: CBC, complete blood count; EDTA, ethylenediaminetetraacetic acid; G6PD, glucose-6-phosphate dehydrogenase; Hb, hemoglobin; Hct, hematocrit; HIV, human immunodeficiency virus; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; PCR, polymerase chain reaction; RBC, red blood cell count; RDW, red cell distribution width; SAO, Southeast Asian Ovalocytosis; VDRL, venereal disease research laboratory test.

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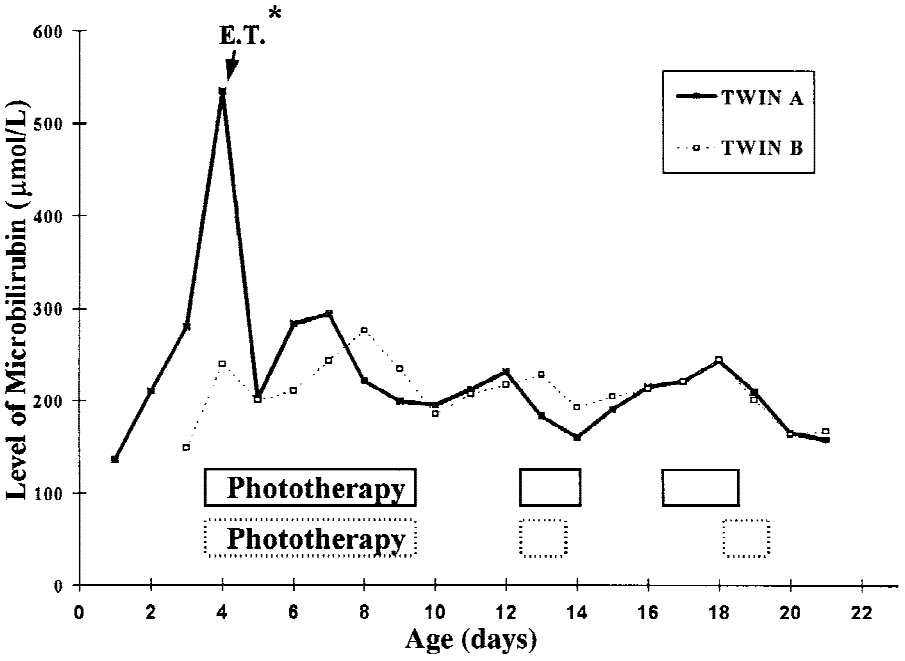

Fig. 1. Plasma microbilirubin in Twin A (SAO with Hb E trait) and Twin B (Hb E trait) E.T.*, exchange blood transfusion.

When 2 days old, twin A became mildly jaundiced but neither the liver nor spleen were palpable. The following laboratory results were obtained: Hct 55%; microbilirubin 136.8 μmol/L (8 mg/dl); ABO and Rh compatible with mother (blood gr O and Rh positive); direct and indirect Coombs' test were negative. One day later the total bilirubin rose to 315 μmol/L (18.42 mg/dl) with a conjugated fraction of 9.4 μmol/L (0.55 mg/dl). CBC showed Hb 15 g/dl; Hct 45%; WBC 15×10^9/L with 63% of neutrophils, 24% of lymphocytes, 11% of monocytes, and 2% of eosinophils; platelet adequate; RBC 3.66×10^{12}/L, MCV 104 fl, MCH 35.7 pg, MCHC 34.4 g/dl, reticulocytes 6.1%; and a peripheral blood film showed mild anisopoikilocytosis, ovalocytosis, and polychromasia with an occasional microspherocyte. No inclusion bodies were seen on examination of the RBC film. Screening test for G6PD enzyme deficiency was normal. Phototherapy was given and the level of microbilirubin was monitored as shown in Figure 1. After 6 hr of phototherapy, the microbilirubin level was still elevated at 451.4 μmol/L (26.4 mg/dl). Exchange blood transfusion was done by 220 ml of O, Rh positive packed RBC reconstituted with 180 ml of O, Rh positive fresh plasma at the age of 4 days. The Hct value prior to exchange blood transfusion was 38% and microbilirubin was 535.2 μmol/L (31.3 mg/dl). The Hct was 53% and microbilirubin was 239.4 μmol/L (14 mg/dl) after exchange blood transfusion. Hb electrophoresis was performed prior to exchange blood transfusion and showed $AFA_2(E)$ with a level of $A_2(E)$ of 2.29%. Phototherapy was continued after exchange blood transfusion and the level of microbilirubin monitored (Fig. 1). The patient showed no abnormality with only mild jaundice and was discharged at the age of 21 days with a Hct of 33% and microbilirubin level of 158.2 μmol/L (9.25 mg/dl). When 6 weeks old he was progressing well with mild pallor and no obvious jaundice. Laboratory investigations showed Hb 7.6 g/dl; Hct 21%; WBC 12.2×10^9/L with N 15%, L 73%, M 6%, E 4% and atypical lymphocytes 2%, RBC 2.65×10^{12}/L; MCV 80 fl; MCH 29 pg; MCHC 36.1 g/dl; reticulocytes 4.8%; and a peripheral blood film showing moderate anisopoikilocytosis, marked ovalocytosis. He was doing well and was seen again at the age of 4 years and showed no abnormalities. Laboratory studies showed Hb 10.7 g/dl; Hct 33%; RBC 5.18×10^{12}/L; MCV 64 fl; MCH 20.7 pg; MCHC 32.1 g/dl; red cell distribution width (RDW) 18.4%; reticulocytes 2.9%; a peripheral blood film showing markedly ovalocytosis and mild anisopoikilocytosis. Hb electrophoresis showed Hb A 74% and Hb E 26%.

Twin B was found to have mild jaundice at the age of 3 days. Neither the liver nor spleen was palpable. The following laboratory results were obtained: Hct 56%; microbilirubin 150.5 μmol/L (8.8 mg/dl); ABO and Rh compatible with mother; direct and indirect Coombs' test negative. CBC showed Hb 16.9 g/dl; Hct 52%; WBC 15.1×10^9/L with N 66%, L 22%, M 10%, E 1%, B 1%, reticulocytes 8.1%; a peripheral blood film showed mild anisopoikilocytosis with an occasional microspherocyte. No inclusion bodies were seen on examination of the RBC film. Screening test for G6PD enzyme deficiency was normal. One day later the microbilirubin rose to 253.1 μmol/L (14.8 mg/dl). Phototherapy was given and microbilirubin levels were monitored (Fig. 1). The mi-

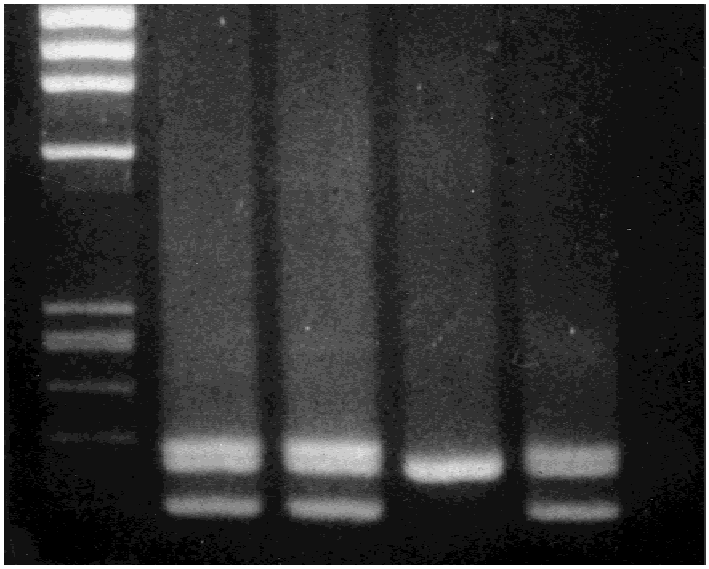

Fig. 2. Amplification of genomic DNA encompassing exon 11 of erythrocyte band 3 gene. Lane 1 is the marker (*Hae*III-digested ϕX 174 phage DNA). Only one band corresponding to 175 bp was visualized after amplification of normal DNA (lane 4). Two separate bands were visualized in the same pattern in lanes 2 (patient's father), 3 (patient) and 5 (amplified product from SAO subject who had 27 bp deletion by DNA sequencing).

crobilirubin level was highest at the level of 273.6 μmol/L (16 mg/dl) at the age of 8 days. She responded well to phototherapy and did not need exchange blood transfusion. She showed no abnormalities with only mild jaundice and was discharged at the age of 21 days with a Hct of 47% and microbilirubin of 166.7 μmol/L (9.75 mg/dl). When 6 weeks old she was progressing well with no obvious jaundice. Laboratory studies showed: Hb 11.2 g/dl; Hct 32%, RBC 3.66×10^{12}/L; MCV 88 fl; MCH 30.6 pg; MCHC 34.6 g/dl; reticulocytes 1.1%; a peripheral blood film showed mild anisopoikilocytosis with no ovalocytosis. At the age of 8 days, Hb electrophoresis showed $AFA_2(E)$ with the level of Hb $A_2(E)$ at 3.37%. She was doing well when seen again at the age of 4 years and showed no abnormalities. Laboratory investigations showed Hb 11.1 g/dl; Hct 35%; RBC 3.01×10^{12}/L; MCV 69 fl; MCH 22.2 pg; MCHC 31.8 g/dl; RDW 14.9%; reticulocytes 2.9%; a peripheral blood film showed mild anisopoikilocytosis. Hb electrophoresis showed Hb A 74% and Hb E 26%.

The twins' father's laboratory studies showed: Hb 13.6 g/dl; Hct 40%; RBC 5.2×10^{12}/L; MCV 76 fl; MCH 26.2 pg; MCHC 34.4 g/dl; RDW 14.6%; reticulocytes 1.7%; a peripheral blood film showing marked ovalocytosis and mild anisopoikilocytosis, which is a typical feature of SAO. Hb electrophoresis showed Hb A 73% and Hb E 27%.

The twins' mother's laboratory studies showed: Hb 13 g/dl; Hct 39%; RBC 4.33×10^{12}/L; MCV 89 fl, MCH 30.3 pg; MCHC 33.7 g/dl; RDW 11.9%; reticulocytes 3.5%; a peripheral blood film showed normal RBC. Her Hb electrophoresis showed Hb A 97.5% and Hb A_2 2.5%.

DNA Analysis

Blood from twin A and his father was collected in sterile tubes containing EDTA. Buffy coat was isolated from whole blood, kept frozen and shipped on ice from Songkla to Japan. Genomic DNA was extracted from buffy coat by standard phenol/chloroform extraction method and used as a template for PCR. PCR amplification of 175 base pairs (bp) long region spreading from nucleotide 1098 to 1272 of erythroid band 3 protein cDNA was performed by using a set of primers [6]. P 198 (5′-GGGCCCAGATGACCCTCTGC-3′; bases 1098–1117) and P 199 (5′-GCCGAAGGTGATGGCGGGTG-3′; bases 1272–1253). The PCR reaction contained 0.5 μM of each primer, 50 ng of DNA template, 200 μM of each dNTP, 1.5 mM $MgCl_2$, 10 mM Tris-HCl (pH 8.3), 50 mM KCl and 1 unit of Takara Taq DNA polymerase (Takara Shuzo Co., Kyoto, Japan). The total reaction mixture was 20 μL, and each reaction was subjected to 30 cycles of 1 min at 94°C, 1 min at 60°C, and 1 min at 72°C by using a Gene AmpPCR system 9600 (Perkin-Elmer Cetus, Emeryville, CA). The amplified DNA fragment was electrophoresed in a 3% agarose gel and stained with ethidium bromide along with low molecular size DNA standards (*Hae*III digested ϕX 174 phage DNA) (Takara Biomedicals). From normal DNA only one band corresponding to 175 bp length was visualized (Fig. 2). As a reference, a DNA sample from an Indonesian ovalocytosis patient who was identified to have 27 nucleotide deletion in the erythroid band 3 gene was used

[7]. Our reported case and his father had an additional smaller band, the same as that found in the reference DNA (148 bp) [7] (Fig. 2). However, there was a slow moving band above the 175 bp band. Sequencing of this band disclosed that this is a mixture of normal and deletion-containing fragments (data not shown). This was identified to be a heteroduplex. This kind of duplex formation is not uncommon [8]. These findings showed that our patient and his father had 27 bp deletion in the erythroid band 3 gene.

DISCUSSION

Although there have been few reported cases of SAO associated with neonatal hyperbilirubinemia [9,10], Honig et al. [9] reported an infant of Philippine parents who became anemic after birth but the authors gave no information about RBC morphology. The infant's serum bilirubin reached 20 mg/dl on the seventh day, at which time the liver and spleen were enlarged. It is possible but not proved that the infant had ABO hemolytic disease of the newborn as well as ovalocytosis [9]. Harrison et al. [10] reported an infant of a New Guinea mother who had SAO. The infant had moderate jaundice at the age of 2 days and blood film showed moderate polychromasia and anisocytosis with occasional microspherocytes, which suggested evidence of increased hemolysis which was not associated with hemolytic disease of the newborn. At the age of 6 weeks, the infant's Hb was 12.7 g/dl; reticulocytes 3.9%, and a peripheral blood film showed typical feature of SAO. At the age of 10 weeks, the Hb was 10.7 g/dl with 7.2% reticulocytes. At 20 weeks of age, the infant had splenomegaly and the Hb was 11.3 g/dl with 4.8% reticulocytes. The mother's blood film showed typical feature of SAO with Hb 14.8 g/dl and reticulocytes 5.2%.

The presented case is twin A of a dizygotic twin, with premature birth and both twins having Hb E heterozygosity. The causes of hyperbilirubinemia in both infants may be due to prematurity which resulted from delayed hepatic bilirubin uptake [11]. Both infants have the same blood group O, Rh positive and their birth weights were quite similar. Their mother had a good antenatal care with negative test for HIV antibody, VDRL, and hepatitis B antigen and antibody. Twin B, who did not have SAO, needed only phototherapy to lower the bilirubin level. But twin A, who had SAO, had rapidly rising bilirubin level and needed phototherapy and exchange blood transfusion to lower the bilirubin concentration. Our findings suggested that SAO played a role in increasing hemolysis and hyperbilirubinemia in twin A. Hb E heterozygosity does not cause hemolysis and hyperbilirubinemia. Among Malayan Aborigines, the frequency of SAO is high, about 12.3%. This ethnic group also has a high frequency of Hb E heterozygosity associated with SAO, which is about 15% [12]. However, there are no reports concerning neonatal hyperbilirubinemia among this ethnic group. Our presented case received a deletion of nine codons or 27 bp in the erythroid band 3 gene and Hb E heterozygosity from his father who had hyperbilirubinmia and needed exchange blood transfusion and phototherapy. No hepatosplenomegaly was detected in this infant.

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